新品番 # BLM-200
TM

LinKit

BIO-LINK

ANTIBODY & PROTEIN BIOTINYLATION

Instruction Protocol
100-200ug

PRODUCT CODE BLM200

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旧品番
ISP-9

LinKit ™ Bio-Link (Code BLM200)

PROTOCOL CONTENTS

A. G50 Buffer Exchange Columns, 3 columns. Store at +4°C - +25°C. **HAZARDOUS,** contains 0.05% sodium azide as a preservative during shipment. Avoid contact with skin and eyes or swallowing. In the event of contamination, seek immediate medical advice.

B. Biotinylation Buffer, 1 x 125ml. Store at +4°C - +25°C.

C. Reaction Tube, 3 tubes with magnetic stirrer bars. Store at +4°C - +25°C.

D. Storage Buffer, 1 x 125ml. Store at +4°C - +25°C. **HAZARDOUS,** contains 0.05% sodium azide as a preservative during shipment. Avoid contact with skin and eyes or swallowing. In the event of contamination, seek immediate medical advice.

E. Solvent for Biotinylation Reagent, 3 x 1.25ml. Store at +4°C - +25°C.

F. Biotin Labelling Reagent, 3 x 1mg. Store at -20°C.

G. Collection Tube, 3 tubes. Store at +4°C - +25°C.

Store Biotin Labelling Reagent at -20°C.

Store the remaining kit at +4 to +25°C, but do not freeze.

If any packs are damaged or bottles appear to have leaked, do not use the items, but contact ISL for advice.

This kit is supplied for Research use only. ISL will not accept responsibility for misuse of the Protocol components. This kit contains glass items which should be handled with due care.

1. The Protocol contains sufficient reagents for 3 antibody labellings, though it can equally be used for other complex proteins of >75kD (Additional formats are available for low molecular weight proteins or antibody fragments).

2. Antibody samples for labelling should be at a concentration of 0.1mg/ml or 0.2mg/ml and free from stabilising proteins such as bovine serum albumin. Recovery will be in the region of 95%.

3. The labelling procedure takes 5 hours to complete. First the antibody preparation is transferred to biotinylation buffer. The biotin label is added and the reaction allowed to proceed for 4 hours at room temperature. The biotinylated antibody preparation is then transferred to phosphate buffered saline containing 0.1% sodium azide preservative, for storage and subsequent use.

4. The labelling ratio obtained should be approximately 20 biotins per antibody. This ratio is dependant on individual antibodies and the number of biotin binding lysine residues they contain.

THE PROTOCOL

You are advised to wear safety glasses and gloves during and after use of the Biotinylation Reagents and Solvent.

A. Exchange to Biotinylation Buffer.

1. Unpack one filtration column **(A)**. Remove the upper cap and pour off the excess buffer. Place the column vertically in a clamp stand, remove the outlet cap and allow any excess buffer to drain to waste.

2. Add 15ml of biotinylation buffer **(B)** to the column and allow the column to drain to waste. The flow rate should be approximately a drip/sec. If it is considerably slower than this the column outlet may be partially obscured and should be carefully trimmed with scissors or a sharp blade.

3. Add 1ml of antibody sample to the column and allow the column to drain to waste.

4. Place the reaction tube **(C)** at the column outlet to collect the antibody as it is eluted. Add 1.5ml biotinylation buffer **(B)** to the column and collect the sample in the reaction tube.

5. Remove the reaction tube. Add 15ml storage buffer to the reservoir of the column **(D)** and allow to drain whilst continuing with the conjugation. Cap the outlet with the yellow closure supplied once the column has drained.

For <u>0.1mg</u> of antibody

B. Biotin Labelling

The following protocol gives an approximate molar ratio of 20 biotin to each antibody. However, as the reaction site for the biotin occurs in the antibody at Lysine amino acid residues, the antibody to target reactivity of some antibodies may be reduced by this degree of labelling. The biotin to antibody ratio may be changed, if required, by altering the volume of biotin label added at labelling step 2 below. To reduce the ratio, add a smaller volume of biotin label. Greater biotin to antibody labelling ratios can also be produced, if required, by addition of up to 200µl of biotin label at labelling step 2. This high conjugation ratio may lead to reduced antibody reactivity.

1. Immediately before use open one vial of solvent **(E)**, wear gloves for safety. This reagent may solidify at temperatures of < 15°C and should be gently warmed until melted.

2 Using a micropipette, transfer 0.5ml of solvent to the vial containing the biotin label **(F)**. Re-cap the vial of biotin label and stir briefly to dissolve the contents.

3 Return 50µl of dissolved biotin label to the vial of solvent **(E)** and mix thoroughly. Add 50µl of this diluted stock in drops to the antibody sample in the reaction tube **(C)** whilst gently shaking.

4. Re-cap the reaction tube and place on a gentle magnetic stirrer for 4 hours at room temperature.

C. Exchange to Storage Buffer

1. If 50µl or less of biotin reagent was added at step 2 of the labelling stage above, make the reaction volume to 2ml by addition of 450µl of storage buffer.

   If more than 50µl of biotin reagent was added at step 2 of the labelling stage above, less storage buffer will have to be added. To determine the volume of storage buffer required, subtract the volume of biotin reagent added from 500µl.

2. Add 1ml (i.e. half) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

3. Place the collection tube **(G)** at the column outlet to collect the biotin labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample in the collection tube.

4. Add 15ml storage buffer to the column and drain to waste.

5. Add 1ml (i.e. the remainder) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

6. Place the collection tube **(G)** at the column outlet to collect the biotin labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample.

7. Store the biotinylated antibody preparation at 4°C.

For 0.2mg of antibody

B. Biotin Labelling

The following protocol gives an approximate molar ratio of 20 biotin to each antibody. However, as the reaction site for the biotin occurs in the antibody at Lysine amino acid residues, the antibody to target reactivity of some antibodies may be reduced by this degree of labelling. The biotin to antibody ratio may be changed, if required, by altering the volume of biotin label added at labelling step 2 below. To reduce the ratio, add a smaller volume of biotin label. Greater biotin to antibody labelling ratios can also be produced, if required, by addition of up to 200µl of biotin label at labelling step 2. This high conjugation ratio may lead to reduced antibody reactivity.

1. Immediately before use open one vial of solvent **(E)**, wear gloves for safety. This reagent may solidify at temperatures of < 15°C and should be gently warmed until melted.

2 Using a micropipette, transfer 0.3ml of solvent to the vial containing the biotin label **(F)**. Re-cap the vial of biotin label and stir briefly to dissolve the contents.

3 Return 60µl of dissolved biotin label to the vial of solvent **(E)** and mix thoroughly. Add 50µl of this diluted stock in drops to the antibody sample in the reaction tube **(C)** whilst gently shaking.

4. Re-cap the reaction tube and place on a gentle magnetic stirrer for 4 hours at room temperature.

C. Exchange to Storage Buffer

1. If 50µl or less of biotin reagent was added at step 2 of the labelling stage above, make the reaction volume to 2ml by addition of 450µl of storage buffer.

   If more than 50µl of biotin reagent was added at step 2 of the labelling stage above, less storage buffer will have to be added. To determine the volume of storage buffer required, subtract the volume of biotin reagent added from 500µl.

2. Add 1ml (i.e. half) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

3. Place the collection tube **(G)** at the column outlet to collect the biotin labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample in the collection tube.

4. Add 15ml storage buffer to the column and drain to waste.

5. Add 1ml (i.e. the remainder) of the reaction mixture to the buffer exchange column, allowing the buffer that drains from the column to go to waste.

6. Place the collection tube **(G)** at the column outlet to collect the biotin labelled antibody. Add 1.5ml storage buffer to the column and collect the eluted sample.

7. Store the biotinylated antibody preparation at 4°C.

Material Safety Data Sheet

Name: Dimethyl Sulphoxide

Chemical Description: C_2H_6SO, Mw 78.13, clear liquid, solid at <19°C.

Hazards: Harmful if inhaled or swallowed or in contact with the skin. Readily absorbed through the skin and may cause sensitisation.

Handling protection: Wear protective clothing, gloves, glasses and face mask. Do not breath dust or swallow. Do not expose to skin and eyes. Avoid prolonged or repeated exposure

First Aid: Flush eyes and skin with large amounts of water. Remove contaminated clothing. If swallowed, rinse mouth and seek medical advice, If inhaled, remove to fresh air.

Handling/storage: Wear protective clothing, gloves, glasses and face mask. Use only in a chemical fume hood. Store in a cool dry place, keep tightly closed, away from ignition sources.

Avoid: Copper wool, potassium permanganate, trichloroacetic acid, acid halides and halide compounds, strong oxidising/reducing agents.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Absorb into inert material, place in bag or bottle for disposal. Wash up any residual.

Fire precautions: Water, dry powder foam. Wear contained breathing equipment. May emit toxic fumes.

Disposal: Approved disposal service. Mix with combustible solvent and burn in a properly equipped incinerator.

The above information is for guide-line purposes only and may not be fully comprehensive. All products should only be handled by trained personnel. Immune Systems and affiliated companies are not liable for any damage caused in any way by the above material.

Material Safety Data Sheet

Name: Sodium Azide

Chemical Description: NaN_3, Mw 65.01, crystalline solid.

Hazards: Highly toxic. Fatal if inhaled or swallowed or absorbed through the skin. May cause genetic damage. May cause explosions. May give toxic gases.

Handling protection: Wear protective clothing, gloves, glasses and face mask. Do not breath dust or swallow. Do not expose to skin and eyes. Avoid prolonged or repeated exposure

First Aid: Flush eyes and skin with large amounts of water. Remove contaminated clothing. If swallowed, rinse mouth and seek medical advice, If inhaled, remove to fresh air.

Handling/storage: Wear protective clothing, gloves, glasses and face mask. Use only in a chemical fume hood. Store in a cool dry place. Keep tightly closed.

Avoid: Sodium azide may react with heavy metals and metal halides to form explosive products. Avoid acids. May explode when heated.

Spills: Clear the area. Clean up wearing suitable clothing, mask, gloves. Place dry contents in bag or bottle for disposal. Wash up any residual.

Fire precautions: Dry powder only. Wear contained breathing equipment. May emit toxic fumes.

Disposal: Approved disposal service.

The above information is for guide-line purposes only and may not be fully comprehensive. All products should only be handled by trained personnel. Immune Systems and affiliated companies are not liable for any damage caused in any way by the above material.